REMARKS

Based on the following remarks, Applicants respectfully request reconsideration and withdrawal of the outstanding rejections set forth in the Action.

A. *Status of the Claims*

Claims 2-3, 5-6, 8-9, 22-23, and 43-45 are currently pending in the application. New independent claim 45 is sought to be added. All other dependent claims 2-3, 5-6, 8-9, 22-23, and 43-44 have been amended to refer back to new claim 45. Support for these claim amendments can be found, for example, at page 5-6, bridging paragraph. Thus, no new matter is added by way of these amendments and their entry is respectfully requested.

B. *Rejection of Claims Under 35 U.S.C. § 112*

Claims 1-3, 5-6, 8-9, 22-23, 43, and 44 were rejected under 35 U.S.C. § 112, first paragraph as allegedly failing to comply with the written description requirement. Claims 1-3, 5-6, 8-9, 22-23, 43 and 44 were rejected under 35 U.S.C. § 112, second paragraph as being indefinite for allegedly failing to particularly point out and distinctly claim the subject matter which applicants regards as the invention. Applicants respectfully disagree with both rejections. However, without acquiescing to either ground for rejection, and solely for the purposes of expediting allowance, Applicants have cancelled claim 1 and added new claim 45 which no longer recites the limitation "and each of which is disposed between the ninety-five to one-thousand and thirteen primer sets amplifying a given target gene sequence of interest."

Thus, Applicants submit that the specification and currently amended claims are fully compliant with the requirement of 35 U.S.C. § 112, first and second paragraphs, and respectfully request that the claim rejections under 35 U.S.C. § 112 be withdrawn accordingly.

C. *Rejection of Claims Under 35 U.S.C. § 103*

Claims 1-3, 5-6, 8-9, 22-23, and 44 were rejected as allegedly being unpatentable over Unger *et al.* (U.S. Patent No. 7,118,910; "Unger") in view of Wang *et al.* (Science 280:1077-1082 (1998); "Wang"); Ohnishi *et al.* (J. Human Genetics 46:471-477 (2001); "Ohnishi"); Dolganov *et al.* (Genome Research 11:1473-1483 (2001); "Dolganov"); and First *et al.* (U.S. Patent No. 5,776,682; "First"). To the extent that these rejections apply to the claims as amended, Applicants respectfully traverse.

1. The cited references

As a preliminary matter, Applicants respectfully disagree with the Examiner's characterization of the cited references.

Applicants respectfully submit that Unger discloses a device with multiple reaction sites for PCR amplification and suggests that an individual reaction site may be used to perform a multiplex PCR reaction. Unger further suggests that the presence of different amplified products in the multiplex reaction can be detected by including differentially labeled probes in a quantitative multiplexed RT-PCR reaction. See Unger at column 30, lines 25-31.

Thus, unlike the presently claimed invention, Unger discloses a method in which quantification of target sequences is performed in a single multiplex PCR reaction, utilizing primer pairs and corresponding probes, with no further steps. In contrast, the presently claimed

methods involve at least two-steps: a multiplex amplification step followed by a single-plex amplification step.

Similarly, Wang discloses nothing more than a multiplex PCR reaction using multiple PCR primers in a single reaction. Unlike the presently claimed invention, Wang does not disclose the use of probes in the multiplex reaction, or the further steps of aliquoting the products of the multiplex reaction for subsequent real-time PCR quantification of particular target sequences within the aliquots using at least one of the primer pairs and probes used in the multiplex reaction. Instead, Wang labels the products of the multiplex PCR for use as probes on a DNA microarray. See Wang at 1082, footnote 26.

Ohnishi discloses a SNP typing system which utilizes a multiplex PCR reaction, followed by analysis of the multiplex reaction for the presence of amplicons containing particular SNPs in a subsequent Invader assay. In contrast to the presently claimed invention, Ohnishi does not disclose the inclusion of probes in the multiplex amplification. Furthermore, an Invader assay involves the use of oligonucleotides that overlap the site of a SNP. In the presence of a SNP, a single-stranded "flap" is generated and cleaved by a FLAP endonuclease to generate a free oligonucleotide fragment which is then detected; nucleic acid is not amplified by PCR in an Invader assay. Thus, Ohnishi also does not teach a further step of real-time PCR quantification of particular target genes present in a prior multiplex reaction. See Ohnishi Methods and Materials section at pages 471-472.

Dolganov discloses the multiplex PCR amplification of the product of a reverse transcription reaction, followed by gene quantification on aliquots of the multiplex reaction using nested TaqMan primers and probes. Thus, unlike the presently claimed invention, Dolganov, at a minimum, does not disclose the inclusion of probes in the multiplex amplification reaction.

Furthermore, Dolganov does not teach the use of the same primer pairs used in the multiplex amplification reaction for the subsequent polynucleotide quantification step; Dolganov, instead, discloses the use of *nested* primers (*i.e.*, "TMF and TMR are nested Taqman primers") which are different then those used in the multiplex reaction (*i.e.*, "RTF and RTR are forward and reverse primers for RT-PCR"). See Dolganov, legend to Figure 1 on page 1474.

First discloses nothing more than a series of primers to markers on the Y-chromosome that can be pooled together to perform a single multiplex PCR reaction. Unlike the presently claimed invention, First does not disclose the use of probes in the multiplex amplification, or the further step of aliquoting the products from the multiplex reaction for subsequent real-time PCR quantification of particular polynucleotide sequences of interest within the aliquots using at least one of the primer pairs and probes used in the multiplex amplification step. See First at column 14, lines 6-13.

In summary, Unger, Wang, and First each disclose a single multiplex amplification reaction, with no subsequent real time PCR step. Unger discloses the additional feature of including a probe in the multiplex PCR reaction (*i.e.*, a multiplex TaqMan assay). Ohnishi discloses a single multiplex PCR reaction, followed by a non-PCR based Invader assay. Dolganov discloses a non-probe containing multiplex amplification reaction followed by a probe-containing gene specific TaqMan assay, using a different set of nested TaqMan primers and probes.

2. The cited references fail to teach each and every element of the claimed invention.

Applicants respectfully submit that the cited references, alone or in any combination, fail to teach all the elements of the presently claimed invention. Specifically, the references, even if

combined, do not teach or suggest a two step method for analyzing a sample for the presence of a polynucleotide sequence of interest comprising the steps of: (i) amplifying a polynucleotide of interest in the presence of a plurality of different amplification primer pairs and a plurality of oligonucleotide probes; and (ii) amplifying the products of step (i) by dividing the products of step (i) into a plurality of aliquots and performing real-time PCR in the presence of at least one of the primer pairs used in step (i) and at least one of the oligonucleotide probes used in step (i).

This is at least because Dolganov, the only reference which describes a non-multiplexed (e.g., single-plexed) real time PCR quantification step, specifically teaches the use of nested primers, which are separate and distinct from those used in Dolganov's previous multiplex PCR step. See Dolganov, legend to Figure 1 on page 1474.

Thus, because the cited references, either alone and/or in combination, fail to teach all the elements of the claimed invention, a *prima facie* case of obviousness has not been established, and Applicants respectfully request withdrawal of this ground for rejection.

3. The skilled artisan would have no motivation to combine the cited references to arrive at the presently claimed invention.

The MPEP suggests that the key to establishing a *prima facie* case of obviousness is "the clear articulation of the reason(s) why the claimed invention would have been obvious." See MPEP 2142. The Federal Circuit has stated that "rejections on obviousness cannot be sustained by mere conclusory statements; instead, there must be some articulated reasoning with some rational underpinning to support the legal conclusion of obviousness." See In re Kahn, 441, F.3d 977, 988, 78 USPQ2d 1329, 1336 (Fed. Cir. 2006).

Regarding a motivation to combine prior art references, the Supreme Court also recently stated that an Examiner's analysis must be made explicit. See KSR Int'l v. Teleflex Inc., U.S. Supreme Court No. 04-1350 (April 30, 2007). Furthermore, the Supreme Court noted that it can be important to identify a reason to combine elements from different references. *Id.* The Supreme Court further warned that "A factfinder should be aware, of course, of the distortion caused by hindsight bias and must be cautious of arguments reliant upon ex post reasoning." *Id.* at 16, citing Graham, 393 U.S., at 36.

In the present case, even if all the claim elements were taught (which they are not for at least the reasons discussed above), the skilled artisan would have no motivation to combine the cited references to arrive at the presently claimed invention. In particular, in designing a two step method for analyzing a sample for the presence of a target sequence, the skilled artisan would have no motivation to include probes (e.g., TaqMan probes) in both a multiplex amplification step and in a subsequent sequence specific real time PCR step. In fact, the skilled artisan would have been taught away from including probes in both steps by the teachings of references, such as Dolganov, which disclose the use of probes in only the second sequence specific real time PCR step, and not in the multiplex step.

Indeed, when performing such a two step procedure, the skilled artisan would be strongly disinclined to make an already complex multiplex PCR mixture, containing both a low abundance, high complexity template and a large number of different primer pairs, even more complex by adding a large number of probes at this step. This is at least because those of skill in the art at the time of Applicants' invention would have believed that doing so would merely increase the occurrence of unwanted side products. Instead, the skilled artisan would more likely add probes to the subsequent gene specific PCR amplification, when a smaller number of primer

pairs and a template enriched for the target of interest was used (for example, a single primer pair as used in Dolganov). Thus, for example, the skilled artisan would have had no motivation to substitute the probeless multiplex step of Dolganov with the single multiplex PCR reaction of Unger, if their intention was to perform a subsequent sequence specific amplification.

In fact, it required the surprising work of the present inventor to show that a robust assay for sequence analysis relying on the inclusion of probes in both a first multiplex amplification and a subsequent gene specific real time PCR could be developed.

Simply put, one of skill in the art, at the time of Applicants' invention, would not have reasonably been motivated to take a highly complicated reaction (a multiplexed amplification reaction) and make it even more complicated (by adding oligonucleotide probes), as is taught by Applicants' presently claims. Performing such a combination would have been believed to simply produce a lot of unwanted non-specific side products, and, at the time, would have been considered a wasteful use of expensive-to-manufacture oligonucleotide probes.

Thus, Applicants respectfully submit that the Action has failed to articulate a motivation for combining the references because no such motivation would have existed prior to the work of the present inventor. In the absence of a motivation to combine the references, and in fact, a teaching away from such a combination, a *prima facie* case of obviousness can not be made, and on this basis also, Applicants respectfully request withdrawal of this ground for rejection.

4. The skilled artisan would have no reasonable expectation of success to arrive at the presently claimed invention by combining the cited references

The prior art can be modified or combined to reject claims as *prima facie* obvious as long as there is a reasonable expectation of success. See *In re* Merck & Co., Inc., 800 F.2d 1091, 231

USPQ 375 (Fed. Cir. 1986). Obviousness does not require absolute predictability, however, at least some degree of predictability is required. Evidence showing there was no reasonable expectation of success may support a conclusion of nonobviousness. See *In re* Rinehart, 531 F.2d 1048, 189 USPQ 143 (CCPA 1976)

The field of molecular biology is considered an uncertain art. In the context of multiplexed PCR, it is generally recognized that increasing the number of target nucleic acids to be analyzed results in a concomitant increase in the number of undesired side reactions. For example, primer dimer formation can rapidly swamp out the desired amplification reactions, thus polluting a PCR with unwanted side products. To further support the above declaration, Applicants earlier submitted a publication by Rudi *et al.* (hereinafter "Rudi") that corroborates the above characterizations of the knowledge of one of skill in the art. (Rudi *et al.*, Nucleic Acids Research, 2003, Vol. 31, No. 11 e62).

Applicants assert that performing a multiplexed amplification with a plurality of primer pairs, along with a plurality of labeled-oligonucleotide probes suitable for monitoring the amplification reaction as a function of time, each of which is complementary to a region of a different amplified target gene sequence of interest would not have been expected reasonably likely to succeed at the time of Applicants' invention.

For example, those of skill in the art (e.g., Rudi *et al.*), considered multiplex methods to be very involved and laborious and often utilized nuclease-mediated removal of unincorporated primers as a way of minimizing unwanted side reactions. The Examiner is invited again to consider Rudi as one illustrative teaching of those of skill in the art during a time period after Applicants' claimed invention.

It was, in fact, Applicants own discovery that by pooling a large number of single plex quantitative PCR kits (a primer pair and a single oligonucleotide probe in each kit) into a single very large multiplex reaction, and by using, for example, low primer concentrations and a limited number of PCR cycles in a first highly multiplexed PCR, highly quantitative results could be obtained in a second PCR. This second PCR can be a single plex reaction, and can include one of the very same kits (primer pair and oligonucleotide probe) that were present in the initial pooled collection of kits. When this approach was attempted, highly accurate results were surprisingly obtained. These results were truly unexpected, since, like Rudi *et al*, those of skill in the art prior to Applicants' claimed invention, would have understood that multiplexed PCR produces unwanted side products, and that by adding additional nucleic acids (e.g. oligonucleotide probes) to an already complex reaction mixture could potentially make more unwanted side products. Further, oligonucleotide probes are expensive to manufacture and adding them unnecessarily to a reaction would have, at the time, been thought to be economically wasteful. Again, for at least these reasons, there would have been no motivation for performing this combination prior to the work of the present inventor.

Through the Applicants own work a two step method involving highly multiplexed amplification involving the use of a large number of primers as well as a large number of oligonucleotide probes (e.g. TaqMan probes) followed by a single plex PCR reaction was successfully developed. One benefit of the claimed approach is, for example, that it allows pre-existing kits (e.g.-single plex PCR kits containing a primer pair and an oligonucleotide probe) to simply be pooled together to perform the highly multiplexed reaction, and then, those same kits in single-plex form can be used in the second PCR to accurately quantify the target nucleic acid. If desired, no additional kits that lack the oligonucleotide probes need to be manufactured.

When dealing with thousands and thousands of kits, this provides an enormous manufacturing (hence economical) advantage. Further, the ability to accurately perform a two-step method for analysis of polynucleotide sequences of interest as currently claimed is unprecedented.

Thus, Applicants respectfully submit that one of skill in the art at the time of Applicants' invention would have reasonably concluded that adding oligonucleotide probes to an already highly multiplexed PCR would merely produce unwanted side products that could potentially prohibit completion of the desired reaction. In the absence of a reasonable expectation of success those of skill in the art would not have combined the cited references to derive the claimed methods. Hence, a *prima facie* case of obviousness can not be made and on this basis also, Applicants respectfully request withdrawal of this ground for rejection.

D. Conclusion

All of the stated grounds of rejection have been properly traversed, accommodated, or rendered moot. Applicants therefore respectfully request that the Examiner reconsider and withdraw all presently outstanding rejections. Applicants believe that a full and complete reply has been made to the outstanding Office Action and, as such, the present application is in condition for immediate allowance.

If the Examiner believes for any reason that a conversation regarding this application and the currently pending claims would expedite allowance, please feel free to contact the undersigned at the number indicated below.

Respectfully submitted,

/Bernadette M. Perfect/

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